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(54) Title: RHESUS MONKEY NEUROPEPTIDE Y Y2 RECEPTOR

(57) Abstract

This invention describes a novel rhesus monkey receptor having affinity for neuropeptide Y, pancreatic polypeptide, and peptide YY. This invention also encompasses nucleic acids encoding this receptor, or a fragment thereof, as well as methods employing this receptor and the nucleic acid compounds.

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Title

RHESUS MONKEY NEUROPEPTIDE Y Y2 RECEPTOR

Background of the Invention

Neuropeptide Y is a peptide present in the central and peripheral nervous systems. The peptide co-exists with noradrenaline in many neurons and acts as a neurotransmitter per se or synergistically together with noradrenaline. Neuropeptide Y-containing fibers are numerous around arteries in the heart, but are also found around the arteries in the respiratory tract, the gastrointestinal tract, and the genitourinary tract. Neuropeptide Y is also present in the cerebrum with effects on blood pressure, feeding, and the release of different hormones. Alterations in central concentrations of neuropeptide Y have been implicated in the etiology of psychiatric disorders.

Neuropeptide Y was discovered, isolated and sequenced about ten years ago from porcine brain as part of a general screening protocol to discover carboxy-terminal amidated peptides and was named neuropeptide Y due to its isolation form neural tissue and the presence of tyrosine as both the amino and carboxy terminal amino acid. Neuropeptide Y is a member of the pancreatic family of peptides and shares significant sequence homology with pancreatic polypeptide, and peptide YY.

Neuropeptide Y and the other members of its family of peptides all feature a tertiary structure consisting of an N-terminal polyproline helix and an amphiphilic α-helix, connected with a β-turn, creating a hairpin-like loop, which is sometimes referred to as the pancreatic polypeptide (PP) fold. The helices are kept together by hydrophobic interactions. The amidated C-terminal end projects away from the hairpin loop.

Subsequent to its discovery neuropeptide Y was identified as being the most abundant peptide in the central nervous system with widespread distribution including the cortex, brainstem, hippocampus, hypothalamus, amygdala, and thalamus as well as being present in the peripheral nervous system in sympathetic neurons and adrenal chromaffin cells.

Neuropeptide Y seems to fulfill the main neurotransmitter criteria, since it is stored in synaptic granules, is released upon electrical nerve stimulation, and acts at specific receptors. It is clear that neuropeptide Y is an important messenger in its own right, probably in the brain, where neuropeptide Y potently inhibits the activity of adenylate cyclase and induces an increase in the intracellular levels of calcium. Central injection of neuropeptide Y results in blood pressure changes, increased feeding, increased fat storage, elevated blood sugar and insulin, decreased locomotor activity, reduced body temperature, and catalepsy.

Neuropeptide Y (as well as its chemical relatives) acts upon membrane receptors that are dependent on guanine nucleotides, known as G protein-coupled receptors. G proteins are a family of membrane proteins that become activated only after binding guanosine triphosphate. Activated G proteins in turn activate an amplifier enzyme on the inner face of a membrane; the enzyme then converts precursor molecules into second messengers.

Neuropeptide Y appears to interact with a family of closely related receptors. These receptors are generally classified into several subtypes based upon the ability of different tissues and receptors to bind different fragments of neuropeptide Y and the closely related peptide YY. The Y1 receptor subtype appears to be the major vascular neuropeptide Y receptor. The Y2 receptor subtypes can also occur postjunctionally on vascular smooth muscle. The as-yet-unisolated Y3 receptor subtype appears to be neuropeptide Y-specific, not binding peptide YY. This receptor is likely to be present in the adrenal tissues, medulla, heart, and brain stem , among other areas. [For a review of neuropeptide Y and neuropeptide Y receptors, see, e.g., C. Wahlestedt and D. Reis, Annual Review of Pharmacology and Toxicology, 33:309-352 (1993)].

In view of the wide number of clinical maladies associated with an excess of neuropeptide Y and related peptides, the development of neuropeptide Y receptor antagonists will serve to control these clinical conditions. The earliest such receptor antagonists were peptide derivatives. These antagonists proved to be of limited pharmaceutical utility because of their metabolic instability.

The present invention provides an additional receptor from the rhesus monkey neuropeptide Y receptor family, the receptor of the present

invention being designated the Y2 receptor, to those previously known. The characterization and treatment of physiological disorders is hereby furthered.

Summary of the Invention

This invention provides an isolated amino acid compound useful as a receptor for neuropeptide Y and related peptides, said compound comprising the amino acid sequence

```
Met Gly Pro Ile Gly Thr Glu Ala Asp Glu Asn Gln Thr Val Glu Glu
 1               5                  10                  15

Met Lys Val Glu Gln Tyr Gly Pro Gln Thr Thr Pro Arg Gly Glu Leu
            20                  25                  30

Val Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Ile Glu
        35                  40                  45

Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly
    50                  55                  60
Val Ile Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser
65                  70                  75                  80

Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp
                85                  90                  95

Leu Val Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu
            100                 105                 110

Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr
        115                 120                 125

Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile
    130                 135                 140

Ala Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile
145                 150                 155                 160

Ser Lys Arg Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Ile Ser
                165                 170                 175

Ala Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile
            180                 185                 190

Glu Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro
        195                 200                 205

Gly Glu Glu Lys Ser Ile Tyr Gly Thr Val Tyr Ser Leu Ser Ser Leu
    210                 215                 220
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Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr
225                 230                 235                 240

Arg Ile Trp Ser Lys Leu Lys Ser His Val Ser Pro Gly Ala Ala Asn
                245                 250                 255

Asp His Tyr His Gln Arg Arg Gln Lys Thr Thr Lys Met Leu Val Cys
            260                 265                 270

Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln
        275                 280                 285

Leu Ala Val Asp Ile Asp Ser His Val Leu Asp Leu Lys Glu Tyr Lys
    290                 295                 300

Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala
305                 310                 315                 320

Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe
                325                 330                 335

Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu
            340                 345                 350

Val Ser Val Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Arg Lys Asn
        355                 360                 365

Ser Gly Pro Asn Asp Ser Phe Thr Glu Ala Thr Asn Val
    370                 375                 380
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hereinafter designated as SEQ ID NO:2.

The invention also provides an isolated nucleic acid compound that comprises a nucleic acid sequence which encodes the amino acid compounds provided. Particularly this invention provides the isolated nucleic acid compound having the sequence

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TGATTGAGGT ACAAGTTGTA GACTCTTGTG CTGGTTGCAG GCCAAGTGGA ACTGTACTGA      60

AA ATG GGT CCA ATA GGT ACA GAG GCT GAT GAG AAC CAG ACA GTG GAA         107
   Met Gly Pro Ile Gly Thr Glu Ala Asp Glu Asn Gln Thr Val Glu
     1               5                  10                  15

GAA ATG AAG GTG GAA CAA TAT GGG CCA CAA ACC ACT CCT AGA GGT GAA        155
Glu Met Lys Val Glu Gln Tyr Gly Pro Gln Thr Thr Pro Arg Gly Glu
                20                  25                  30

CTG GTC CCT GAT CCT GAG CCA GAG CTT ATA GAT AGT ACC AAG CTG ATT        203
Leu Val Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Ile
            35                  40                  45
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GAG GTA CAA GTT GTC CTC ATA TTG GCC TAT TGC TCC ATC ATC TTG CTT      251
Glu Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu
         50                  55                  60

GGG GTA ATT GGC AAC TCC TTG GTG ATC CAC GTG GTG ATC AAA TTC AAG      299
Gly Val Ile Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys
     65                  70                  75

AGC ATG CGC ACA GTA ACC AAC TTT TTC ATC GCC AAT CTG GCT GTG GCA      347
Ser Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala
 80                  85                  90                  95

GAT CTT GTG GTG AAT ACT CTG TGT CTA CCA TTC ACT CTT ACC TAC ACC      395
Asp Leu Val Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr
                100                 105                 110

TTA ATG GGG GAG TGG AAA ATG GGT CCT GTC CTG TGC CAC CTG GTG CCC      443
Leu Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro
            115                 120                 125

TAT GCA CAG GGC CTG GCA GTA CAA GTA TCC ACA ATC ACC TTG ACA GTA      491
Tyr Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val
        130                 135                 140

ATT GCC CTG GAC CGG CAC AGG TGC ATC GTC TAC CAC CTG GAG AGC AAG      539
Ile Ala Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys
    145                 150                 155

ATC TCC AAG CGT ATC AGC TTC CTG ATT ATT GGC TTG GCC TGG GGC ATC      587
Ile Ser Lys Arg Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Ile
160                 165                 170                 175

AGT GCC CTG CTA GCA AGT CCC CTG GCC ATC TTC CGG GAG TAT TCA CTG      635
Ser Ala Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu
                180                 185                 190

ATT GAG ATC ATT CCG GAT TTT GAG ATT GTG GCC TGT ACT GAA AAA TGG      683
Ile Glu Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp
            195                 200                 205

CCT GGC GAG GAA AAG AGC ATC TAT GGC ACT GTC TAC AGT CTT TCT TCC      731
Pro Gly Glu Glu Lys Ser Ile Tyr Gly Thr Val Tyr Ser Leu Ser Ser
        210                 215                 220

TTG TTG ATC CTG TAC GTT TTG CCT CTG GGC ATA ATA TCA TTT TCC TAC      779
Leu Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr
    225                 230                 235

ACT CGC ATT TGG AGT AAA TTG AAG AGC CAT GTC AGT CCT GGA GCT GCA      827
Thr Arg Ile Trp Ser Lys Leu Lys Ser His Val Ser Pro Gly Ala Ala
240                 245                 250                 255
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AAT GAC CAC TAC CAT CAG CGA AGG CAA AAA ACC ACC AAA ATG CTG GTG      875
Asn Asp His Tyr His Gln Arg Arg Gln Lys Thr Thr Lys Met Leu Val
               260                 265                 270

TGC GTG GTG GTG GTG TTT GCG GTC AGC TGG CTG CCT CTC CAT GCC TTC      923
Cys Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe
            275                 280                 285

CAG CTT GCC GTT GAC ATT GAC AGC CAT GTC CTG GAC CTG AAG GAG TAC      971
Gln Leu Ala Val Asp Ile Asp Ser His Val Leu Asp Leu Lys Glu Tyr
        290                 295                 300

AAA CTC ATC TTC ACA GTG TTC CAC ATC ATC GCC ATG TGC TCC ACT TTT     1019
Lys Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe
    305                 310                 315

GCC AAT CCC CTT CTC TAT GGC TGG ATG AAC AGC AAC TAT AGA AAG GCT     1067
Ala Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala
320                 325                 330                 335

TTC CTC TCT GCC TTC CGC TGT GAG CAG CGG TTG GAT GCC ATT CAC TCT     1115
Phe Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser
               340                 345                 350

GAG GTG TCC GTG ACA TTC AAG GCT AAA AAG AAC CTG GAG GTC AGA AAA     1163
Glu Val Ser Val Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Arg Lys
            355                 360                 365

AAT AGT GGC CCC AAT GAC TCT TTC ACA GAA GCT ACC AAT GTC             1205
Asn Ser Gly Pro Asn Asp Ser Phe Thr Glu Ala Thr Asn Val
        370                 375                 380

TAAGGAAGCT AGGGTGTGAA AATGTATGAA TGAATTCTGA CCAGAGCTAT AAATCTGGTT    1265

GATGGCGGCT CACAAGTGAT AATTGATTTC CCATTTTAAG GAAGAAGAGG ATCTAAATGG    1325

AAGCATCTGC TGTTTAGTTC CTGGAAAACT GGCTGGGAAG AGCCTGTGTG AAAATACTTG    1385

AATTCAAAGA TAAGGCAGCA AAATGGTTTA CTTAACAGTT GGTAGGGTAG TAGGTTGAAT    1445

TAGGAGTAAA AGCAGAGAGA GGTACTTTTG ACTATTTTCC TGGAGTGAAG TAAACTTGAA    1505

CAAGGAATTG GTATTATCAG CATTGCAAAG AGACGGTGGG TAAATAAGTT GATTTTCAGA    1565

TTTCATTAGG ACCTGGATTG GGGAGCTGTG TAGTTCACGG TTCCCTGCTT GGCTGATGAA    1625

AACGTCGCTG AACAAAAATT TCTCCAGGGA GCCACAGGCT CTCCTTCATC ACGTTTTGAT    1685

TTTTTTTGTT AATTCTCTAG ACAAAATCCA TCAAGGAATG CTGCAGGAAA AGATTGCCAG    1745

CTATATGAAT GGCTTCAAGG AACTAAACTG AAACTTGCTA TATAATTAAT ATTTTGGCAG    1805

ACGATAGGGG AACTCCTCAA CACTCAGTGA GCCAATTGTT CTTAAAACCG GTTGCACATT    1865
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TGGTGAAAGT TTCTTCAACT CTGAATCAAA AGCTGAAATT CTCAGAATTG CAGGAAATGC   1925

AAACCATCAT TTAATTTGTA ATTTCAAGTT ACATCTGCTT TATGGAGATA TTTAGATAAC   1985

AAGCATACAA CTTGATAGTT TTATTGTTAT ACCTTTTTGA ACATGTATGA TTTATGTTAT   2045

TATTCCTATT GGAGCTAAGT TTGTCTACAC TAAAATTTAA ATCAGAATAA AGAATAATTT   2105

TTGTGGAAAA AAAAAAAAAA AAAAAAAAAA AAACTCGAG                          2144
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which is hereinafter designated as SEQ ID NO:1.

This invention also provides recombinant nucleic acid vectors comprising nucleic acids encoding SEQ ID NO:2. This invention also encompasses recombinant DNA vectors which comprise the isolated DNA sequence which is SEQ ID NO:1.

The present invention also provides assays for determining the efficacy and adverse reaction profile of agents useful in the treatment or prevention of disorders associated with an excess or deficiency in the amount of neuropeptide Y present.

Detailed Description and Preferred Embodiments

The terms and abbreviations used in this document have their normal meanings unless otherwise designated. For example "_C" refers to degrees Celsius; "N" refers to normal or normality; "mmol" refers to millimole or millimoles; "g" refers to gram or grams; "ml" means milliliter or milliliters; "M" refers to molar or molarity; "µg" refers to microgram or micrograms; and "µl" refers to microliter or microliters.

All nucleic acid sequences, unless otherwise designated, are written in the direction from the 5' end to the 3' end, frequently referred to as "5' to 3'".

All amino acid or protein sequences, unless otherwise designated, are written commencing with the amino terminus ("N-terminus") and concluding with the carboxy terminus ("C-terminus").

"Base pair" or "bp" as used herein refers to DNA or RNA. The abbreviations A,C,G, and T correspond to the 5'-monophosphate forms of the deoxyribonucleosides (deoxy)adenine, (deoxy)cytidine, (deoxy)guanine, and (deoxy)thymine, respectively, when they occur in DNA molecules. The

abbreviations U,C,G, and T correspond to the 5'-monophosphate forms of the ribonucleosides uracil, cytidine, guanine, and thymine, respectively when they occur in RNA molecules. In double stranded DNA, base pair may refer to a partnership of A with T or C with G. In a DNA/RNA, heteroduplex base pair may refer to a partnership of A with U or C with G. (See the definition of "complementary", infra.)

The terms "digestion" or "restriction" of DNA refers to the catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA ("sequence-specific endonucleases"). The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements were used as would be known to one of ordinary skill in the art. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer or can be readily found in the literature.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments. Unless otherwise provided, ligation may be accomplished using known buffers and conditions with a DNA ligase, such as T4 DNA ligase.

The term "plasmid" refers to an extrachromosomal (usually) self-replicating genetic element. Plasmids are generally designated by a lower case "p" preceded and/or followed by letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accordance with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

The term "reading frame" means the nucleotide sequence from which translation occurs "read" in triplets by the translational apparatus of transfer RNA (tRNA) and ribosomes and associated factors, each triplet corresponding to a particular amino acid. A base pair insertion or deletion (termed a frameshift mutation) may result in two different proteins being coded for by the same DNA segment. To insure against this, the triplet codons corresponding to the desired polypeptide must be aligned in multiples of three from the initiation codon, i.e. the correct "reading frame" being maintained.

"Recombinant DNA cloning vector" as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been added.

The term "recombinant DNA expression vector" as used herein refers to any recombinant DNA cloning vector in which a promoter to control transcription of the inserted DNA has been incorporated.

The term "expression vector system" as used herein refers to a recombinant DNA expression vector in combination with one or more trans-acting factors that specifically influence transcription, stability, or replication of the recombinant DNA expression vector. The trans-acting factor may be expressed from a co-transfected plasmid, virus, or other extrachromosomal element, or may be expressed from a gene integrated within the chromosome.

"Transcription" as used herein refers to the process whereby information contained in a nucleotide sequence of DNA is transferred to a complementary RNA sequence.

The term "transfection" as used herein refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, calcium phosphate co-precipitation, and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

The term "transformation" as used herein means the introduction of DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Methods of transforming bacterial and eukaryotic hosts are well known in the art, many of which methods, such as nuclear injection, protoplast fusion or by calcium treatment using calcium chloride are summarized in J. Sambrook, et al., MOLECULAR CLONING: A LABORATORY MANUAL, (1989).

The term "translation" as used herein refers to the process whereby the genetic information of messenger RNA is used to specify and direct the synthesis of a polypeptide chain.

The term "vector" as used herein refers to a nucleic acid compound used for the transformation of cells in gene manipulation bearing polynucleotide sequences corresponding to appropriate protein molecules which when combined with appropriate control sequences confer specific

properties on the host cell to be transformed. Plasmids, viruses, and bacteriophage are suitable vectors. Artificial vectors are constructed by cutting and joining DNA molecules from different sources using restriction enzymes and ligases. The term "vector" as used herein includes Recombinant DNA cloning vectors and Recombinant DNA expression vectors.

The terms "complementary" or "complementarity" as used herein refers to pair of bases, purines and pyrimidines, that associate through hydrogen bonding in double stranded nucleic acid. The following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil.

The term "hybridization" as used herein refers to a process in which a strand of nucleic acid joins with a complementary strand through base pairing. The conditions employed in the hybridization of two non-identical, but very similar, complementary nucleic acids varies with the degree of complementarity of the two strands and the length of the strands. Such techniques and conditions are well known to practitioners in this field.

"Isolated amino acid sequence" refers to any amino acid sequence, however constructed or synthesized, which is locationally distinct from the naturally occurring sequence.

"Isolated DNA compound" refers to any DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location in genomic DNA.

"Isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation.

The term "promoter" refers to a DNA sequence which directs transcription of DNA to RNA.

A "probe" as used herein is a nucleic acid compound or a fragment thereof which hybridizes with a nucleic acid compound which encodes either the entire sequence SEQ ID NO:2, a sequence complementary to SEQ ID NO:2, or a part thereof.

The term "stringency" refers to a set of hybridization conditions which may be varied in order to vary the degree of nucleic acid affinity for other nucleic acid. (See the definition of "hybridization", supra.)

The term "antigenically distinct" as used herein refers to a situation in which antibodies raised against an epitope of the proteins of the present invention, or a fragment thereof, may be used to differentiate between the proteins of the present invention and other neuropeptide Y receptor subtypes. This term may also be employed in the sense that such antibodies may be used to differentiate between the rhesus monkey Y2 receptor protein and analogous proteins derived from other species.

The term "PCR" as used herein refers to the widely-known polymerase chain reaction employing a thermally-stable polymerase.

This invention provides the protein of SEQ ID NO:2, a rhesus monkey neuropeptide Y receptor, designated as a Y2 receptor. [For a review of neuropeptide Y receptors, see, D. Gehlert, Life Sciences, 55:551-562 (1994)]. Traditional receptors of this family have considerable overlap in their binding affinities for neuropeptide Y and peptide YY while pancreatic polypeptide appears to have its own distinct set of receptors. Many, but not all, of the effects of neuropeptide Y can be replicated using peptide YY. The receptor of the present invention, as described infra, has considerable pharmacological overlap between pancreatic polypeptide and peptide YY and less affinity for neuropeptide Y, indicating it belongs to a novel subclass of receptors.

Two subtypes of receptors for neuropeptide Y were initially proposed on the basis of the affinity of the 13-36 fragment of neuropeptide Y using a preparation of the sympathetic nervous system. While these are the best established receptors for neuropeptide Y, a substantial body of evidence exists that there are additional receptor subtypes. The best established is a Y-3 receptor that is responsive to neuropeptide Y, but not to peptide YY. Another recently delineated receptor has been described that binds peptide YY with high affinity and neuropeptide Y with lower affinity. While the pharmacology of the feeding response to neuropeptide Y appears to be Y-1 in nature, a separate "feeding receptor" has been proposed. Until this invention, the Y-1 receptor was the only one that had been successfully cloned to date. The following paragraphs summarize the available information on the known neuropeptide Y receptor subtypes and their potential role in physiological function.

Y-1 Receptor

The Y-1 receptor is the best characterized receptor for neuropeptide Y. This receptor is generally considered to be postsynaptic and mediates many of the known actions of neuropeptide Y in the periphery. Originally, this receptor was described as having poor affinity for C-terminal fragments of neuropeptide Y, such as the 13-36 fragment, but interacts with the full length neuropeptide Y and peptide YY with equal affinity. L. Selbie, et al., Patent Cooperation Treaty publication WO 93/09227, published May 13, 1993; C. Wahlestedt, et al., Regulatory Peptides, 13:307-318 (1986); C. Wahlestedt, et al., NEURONAL MESSENGERS IN VASCULAR FUNCTION, 231-241 (Nobin, et al., eds. 1987). Substitution of the amino acid at position 34 with a proline (Pro^{34}) results in a protein which is specific for the Y-1 receptor. E.K. Potter, et al., European Journal of Pharmacology, 193:15-19 (1991). This tool has been used to establish a role for the Y-1 receptor in a variety of functions. The receptor is thought to be coupled to adenylate cyclase in an inhibitory manner in cerebral cortex, vascular smooth muscle cells, and SK-N-MC. [For a review, see, B.J. McDermott, et al., Cardiovascular Research, 27:893-905 (1993)]. This action is prevented by application of pertussis toxin confirming the role of a G-protein coupled receptor. The Y-1 receptor mediates the mobilization of intracellular calcium in a porcine vascular smooth muscle cells and human erythroleukemia cells.

The cloned human Y-1 receptor can couple to either phosphotidylinositol hydrolysis or the inhibition of adenylate cyclase, depending on the type of cell in which the receptor is expressed. H. Herzog, et al., Proceedings of the National Academy of Sciences (USA), 89:5794-5798 (1992). The Y-1 receptor has been reported to couple to either second messenger system when studied using tissue preparations or cell lines naturally expressing the receptor. D. Gehlert, supra, at 553. The Y-1 receptor cannot, therefore, be distinguished solely on the basis of coupling to a single second messenger.

Y-2 Receptor

As with the Y-1 receptor, this receptor subtype was first delineated using vascular preparations. Pharmacologically, the Y-2 receptor is distinguished from Y-1 by exhibiting affinity for C-terminal fragments of

neuropeptide Y. The receptor is most often differentiated by the use of neuropeptide Y(13-36), though the 3-36 fragment of neuropeptide Y and peptide YY provides improved affinity and selectivity. Y. Dumont, et al., Society for Neuroscience Abstracts, 19:726 (1993). Like Y-1 receptor, this receptor is coupled to the inhibition of adenylate cyclase, though in some preparations it may not be sensitive to pertussis toxin. The Y-2 receptor was found to reduce the intracellular levels of clacium in the synspse by selective inhibition of N-type calcium channels. Like the Y-1 receptor, the Y-2 receptor may exhibit differential coupling to second messengers.

The Y-2 receptors are found in a variety of brain regions, including the hippocampus, substantia nigra-lateralis, thalamus, hypothalamus, and brainstem. In the periphery, Y-2 is found in the peripheral nervous system, such as sympathetic, parasympathetic, and sensory neurons. In all these tissues, Y-2 receptors mediate a decrease in the release of neurotransmitters.

Y-3 Receptor

This receptor is the newest and least studied of the established neuropeptide Y receptor subtypes. While neuropeptide Y is a fully efficacious agonist at this receptor population, peptide YY is weakly efficacious. This pharmacological property is used to define this receptor. A receptor that has similar pharmacology to the Y-3 receptor has been identified in the CA3 region of the hippocampus using electrophysiological techniques. This receptor may potentiate the excitatory response of these neurons to N-methyl-D-aspartate (NMDA). F.P. Monnet, et al., European Journal of Pharmacology, 182:207-208 (1990).

The presence of this receptor is best established in the rhesus monkey brainstem, specifically in the nucleus tractus solitarius. Application of neuropeptide Y to this region produces a dose-dependent reduction in blood pressure and heart rate. This area of the brain also may have significant contributions from the Y-1 and Y-2 receptor. Neuropeptide Y also inhibits the acetylcholine-induced release of catecholamines from the adrenal medulla, presumably through a Y-3 receptor. C. Wahlestedt, et al., Life Sciences, 50:PL7-PL14 (1992).

Peptide YY Preferring Receptor

A fourth receptor has been described that exhibits a modest preference for peptide YY over neuropeptide Y. This receptor was first described in the rhesus monkey small intestine as having a 5-10 fold higher affinity for peptide YY over neuropeptide Y. M. Laburthe, et al., Endocrinology, 118:1910-1917 (1986). Subsequently, this receptor was found in the adipocyte and a kidney proximal tubule cell line. This receptor is coupled in an inhibitory manner to adenylate cyclase and is sensitive to pertussis toxin.

In the intestine, this receptor produces a potent inhibition of fluid and electrolyte secretion. The receptor is localized to the crypt cells where intestinal chloride secretion is believed to take place. The peptide YY preferring receptor in adipocytes mediates a reduction in lipolysis by way of a cyclic adenosine monophosphate (cAMP)-dependent mechanism.

"Feeding Receptor"

One of the earliest discovered central effects of neuropeptide Y was a profound increase in food intake that was observed following the hypothalmic administration of the peptide to rats. The response was greatest when the peptide was infused into the perifornical region of the hypothalamus. B.G. Stanley, et al., Brain Research, 604:304-317 (1993). While the pharmacology of this response resembled the Y-1 receptor, the 2-36 fragment of neuropeptide Y was significantly more potent than neuropeptide Y. In addition, intracerebroventricular neuropeptide Y(2-36) fully stimulates feeding, but does not reduce body temperature as does full length neuropeptide Y. F.B. Jolicoeur, et al., Brain Research Bulletin, 26:309-311 (1991).

The receptors of the present invention are believed to potentiate central nervous system responses and is, therefore, an important target for pharmaceutical purposes. The receptor of the present invention will be useful in identifying compounds useful in the treatment or prevention of conditions associated with an excess of neuropeptide Y. The term "physiological disorder associated with an inappropriate amount of

neuropeptide Y, peptide YY, or pancreatic polypeptide" encompasses those disorders associated with an inappropriate stimulation of a receptor of these neuropeptides, regardless of the actual amount of the neuropeptide present in the locale.

These physiological disorders include:

disorders or diseases pertaining to the heart, blood vessels or the renal system, such as vasospasm, heart failure, shock, cardiac hypertrophy, increased blood pressure, angina, myocardial infarction, sudden cardiac death, arrythmia, peripheral vascular disease, and abnormal renal conditions such as impaired flow of fluid, abnormal mass transport, or renal failure;

conditions related to increased sympathetic nerve activity for example, during or after coronary artery surgery, and operations and surgery in the gastrointestinal tract;

cerebral diseases and diseases related to the central nervous system, such as cerebral infarction, neurodegeneration, epilepsy, stroke, and conditions related to stroke, cerebral vasospasm and hemorrhage, depression, anxiety, schizophrenia, and dementia;

conditions related to pain or nociception;

diseases related to abnormal gastrointestinal motility and secretion, such as different forms of ileus, urinary incontinence, and Crohn's disease;

abnormal drink and food intake disorders, such as obesity, anorexia, bulimia, and metabolic disorders;

diseases related to sexual dysfunction and reproductive disorders;

conditions or disorders associated with inflammation;

respiratory diseases, such as asthma and conditions related to asthma and bronchoconstriction; and

diseases related to abnormal hormone release, such as leutinizing hormone, growth hormone, insulin, and prolactin.

Skilled artisans will recognize that the proteins of the present invention can be synthesized by a number of different methods. All of the amino acid compounds of the invention can be made by chemical methods well known in the art, including solid phase peptide synthesis, or

recombinant methods. Both methods are described in U.S. Patent 4,617,149, herein incorporated by reference.

The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area. See, e.g., H. Dugas and C. Penney, BIOORGANIC CHEMISTRY, (1981) Springer-Verlag, New York, pgs. 54-92. For examples, peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (commercially available from Applied Biosystems, Foster City California) and synthesis cycles supplied by Applied Biosystems. Protected amino acids, such as *t*-butoxycarbonyl-protected amino acids, and other reagents are commercially available from many chemical supply houses.

Sequential *t*-butoxycarbonyl chemistry using double couple protocols are applied to the starting *p*-methyl benzhydryl amine resins for the production of C-terminal carboxamides. For the production of C-terminal acids , the corresponding pyridine-2-aldoxime methiodide resin is used. Asparagine, glutamine, and arginine are coupled using preformed hydroxy benzotriazole esters. The following side chain protection may be used:

Arg, Tosyl
Asp, cyclohexyl
Glu, cyclohexyl
Ser, Benzyl
Thr, Benzyl
Tyr, 4-bromo carbobenzoxy

Removal of the *t*-butoxycarbonyl moiety (deprotection) may be accomplished with trifluoroacetic acid (TFA) in methylene chloride. Following completion of the synthesis the peptides may be deprotected and cleaved from the resin with anhydrous hydrogen fluoride containing 10% meta-cresol. Cleavage of the side chain protecting group(s) and of the peptide from the resin is carried out at zero degrees centigrade or below, preferably -20_C for thirty minutes followed by thirty minutes at 0_C.

After removal of the hydrogen fluoride, the peptide/resin is washed with ether, and the peptide extracted with glacial acetic acid and then lyophilized. Purification is accomplished by size-exclusion chromatography on a Sephadex G-10 (Pharmacia) column in 10% acetic acid.

The proteins of the present invention may also be produced by recombinant methods. Recombinant methods are preferred if a high yield is

desired. A general method for the construction of any desired DNA sequence is provided in J. Brown, et al., Methods in Enzymology, 68:109 (1979). See also, J. Sambrook, et al., supra.

The basic steps in the recombinant production of desired proteins are:

a) construction of a synthetic or semi-synthetic DNA encoding the protein of interest;

b) integrating said DNA into an expression vector in a manner suitable for the expression of the protein of interest, either alone or as a fusion protein;

c) transforming an appropriate eukaryotic or prokaryotic host cell with said expression vector,

d) culturing said transformed or transfected host cell in a manner to express the protein of interest; and

e) recovering and purifying the recombinantly produced protein of interest.

In general, prokaryotes are used for cloning of DNA sequences in constructing the vectors of this invention. Prokaryotes may also be employed in the production of the protein of interest. For example, the Escherichia coli K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of E. coli which may be used (and their relevant genotypes) include the following.

Strain	Genotype
DH5α	F^- (φ80dlacZΔM15), Δ(lacZYA-argF)U169 supE44, λ^-, hsdR17(r_K^-, m_K^+), recA1, endA1, gyrA96, thi-1, relA1

HB101	supE44, hsdS20(r_B^- m_B^-), recA13, ara-14, $proA_2$ lacY1, galK2, rpsL20, xyl-5, mtl-1, mcrB, mrr
JM109	recA1. e14-(mcrA), supE44, endA1, hsdR17(r_K^-, m_K^+), gyrA96, relA1, thi-1, Æ(lac-proAB), F'[traD36. proAB+ $lacI^q$,lacZÆM15]
RR1	supE44, hsdS20(r_B^- m_B^-), ara-14 $proA_2$. lacY1, galK2, rpsL20, xyl-5, mtl-5
χ1776	F-, ton, A53, dapD8, minA1, supE42 (glnV42), Δ(gal-uvrB)40. minB2, rfb-2, $gyrA_25$, thyA142, oms-2, metC65, oms-1, Δ(bioH-asd)29, cycB2, cycA1, hsdR2, λ-
294	endA, thi-, hsr-, hsm_k^+ (U.S. Patent 4,366,246)
LE392	F-, hsdR514 (r-m-). supE44, supF58, lacY1. or Δlac(I-Y)6, galK2, glaT22, metB1, trpR55, λ-

These strains are all commercially available from suppliers such as: Bethesda Research Laboratories, Gaithersburg, Maryland 20877 and Stratagene Cloning Systems, La Jolla, California 92037; or are readily available to the poblic from sources such as the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 10852-1776.

Except where otherwise noted, these bacterial strains can be used interchangeably. The genotypes listed are illustrative of many of the desired characteristics for choosing a bacterial host and are not meant to limit the invention in any way. The genotype designations are in accordance with standard nomenclature. See, for example, J. Sambrook, et al., supra. A preferred strain of E. coli employed in the cloning and expression of the genes

of this invention is RV308, which is available from the ATCC under accession number ATCC 31608, and is described in United States Patent 4,551,433, issued November 5, 1985.

In addition to the strains of E. coli discussed supra, bacilli such as Bacillus subtilis, other enterobacteriaceae such as Salmonella typhimurium or Serratia marcescans, and various Pseudomonas species may be used. In addition to these gram-negative bacteria, other bacteria, especially Streptomyces, spp., may be employed in the prokaryotic cloning and expression of the proteins of this invention.

Promoters suitable for use with prokaryotic hosts include the β-lactamase [vector pGX2907 (ATCC 39344) contains the replicon and β-lactamase gene] and lactose promoter systems [Chang et al., Nature (London), 275:615 (1978); and Goeddel et al., Nature (London), 281:544 (1979)], alkaline phosphatase, the tryptophan (trp) promoter system [vector pATH1 (ATCC 37695) is designed to facilitate expression of an open reading frame as a trpE fusion protein under control of the trp promoter] and hybrid promoters such as the tac promoter (isolatable from plasmid pDR540 ATCC-37282). However, other functional bacterial promoters, whose nucleotide sequences are generally known, enable one of skill in the art to ligate them to DNA encoding the proteins of the instant invention using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

The proteins of this invention may be synthesized either by direct expression or as a fusion protein comprising the protein of interest as a translational fusion with another protein or peptide which may be removable by enzymatic or chemical cleavage. It is often observed in the production of certain peptides in recombinant systems that expression as a fusion protein prolongs the lifespan, increases the yield of the desired peptide, or provides a convenient means of purifying the protein of interest. A variety of peptidases (e.g. trypsin) which cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (e.g. diaminopeptidase) of the peptide chain are known. Furthermore, particular chemicals (e.g. cyanogen bromide) will cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and

synthetic or semi-synthetic coding sequence if recombinant means are employed) to incorporate site-specific internal cleavage sites. See e.g., P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13 in PROTEIN PURIFICATION: FROM MOLECULAR MECHANISMS TO LARGE SCALE PROCESSES, American Chemical Society, Washington, D.C. (1990).

In addition to cloning and expressing the genes of interest in the prokaryotic systems discussed above, the proteins of the present invention may also be produced in eukaryotic systems. The present invention is not limited to use in a particular eukaryotic host cell. A variety of eukaryotic host cells are available from depositories such as the American Type Culture Collection (ATCC) and are suitable for use with the vectors of the present invention. The choice of a particular host cell depends to some extent on the particular expression vector used to drive expression of the rhesus monkey neuropeptide Y-like receptor-encoding nucleic acids of the present invention. Exemplary host cells suitable for use in the present invention are listed in Table I

Table I

Host Cell	Origin	Source
HepG-2	Human Liver Hepatoblastoma	ATCC HB 8065
CV-1	African Green Monkey Kidney	ATCC CCL 70
LLC-MK_2	Rhesus Monkey Kidney	ATCC CCL 7
3T3	Mouse Embryo Fibroblasts	ATCC CCL 92
CHO-K1	Chinese Hamster Ovary	ATCC CCL 61
HeLa	Human Cervix Epitheloid	ATCC CCL 2
RPMI8226	Human Myeloma	ATCC CCL 155
H4IIEC3	Rat Hepatoma	ATCC CCL 1600
C127I	Mouse Fibroblast	ATCC CCL 1616
293	Human Embyronal Kidney	ATCC CRL 1573
HS-Sultan	Human Plasma Cell Plasmocytoma	ATCC CCL 1484
BHK-21	Baby Hamster Kidney	ATCC CCL 10

An especially preferred cell line employed in this invention is the widely available cell line AV12-664 (hereinafter "AV12"). This cell line is available from the American Type Culture Collection under the accession number ATCC CRL 9595. The AV12 cell line was constructed by injecting a Syrian hamster in the scruff of the neck with human adenovirus 12 and isolating cells from the resulting tumor.

A wide variety of vectors, some of which are discussed below, exists for the transformation of such mammalian host cells, but the specific vectors described herein are in no way intended to limit the scope of the present invention.

The pSV2-type vectors comprise segments of the simian virus 40 (SV40) genome that constitute a defined eukaryotic transcription unit-promoter, intervening sequence, and polyadenylation site. In the absence of the SV40 T antigen, the plasmid pSV2-type vectors transform mammalian and other eukaryotic host cells by integrating into the host cell chromosomal DNA. A large number of plasmid pSV2-type vectors have been constructed, such as plasmid pSV2-gpt, pSV2-neo, pSV2-dhfr, pSV2-hyg, and pSV2-β-globin, in which the SV40 promoter drives transcription of an inserted gene. These vectors are suitable for use with the coding sequences of the present

invention and are widely available from sources such as the ATCC or the Northern Regional Research Laboratory (NRRL), 1815 N. University Street, Peoria, Illinois, 61604.

The plasmid pSV2-dhfr (ATCC 37146) comprises a murine dihydrofolate reductase (dhfr) gene under the control of the SV40 early promoter. Under the appropriate conditions, the dhfr gene is known to be amplified, or copied, in the host chromosome. This amplification can result in the amplification of closely-associated DNA sequences and can, therefore, be used to increase production of a protein of interest. See, e.g., J. Schimke, Cell, 35:705-713 (1984).

Plasmids constructed for expression of the proteins of the present invention in mammalian and other eukaryotic host cells can utilize a wide variety of promoters. The present invention is in no way limited to the use of the particular promoters exemplified herein. Promoters such as the SV40 late promoter, promoters from eukaryotic genes, such as, for example, the estrogen-inducible chicken ovalbumin gene, the interferon genes, the gluco-corticoid-inducible tyrosine aminotransferase gene, and the thymidine kinase gene, and the major early and late adenovirus genes can be readily isolated and modified to express the genes of the present invention. Eukaryotic promoters can also be used in tandem to drive expression of a coding sequence of this invention. Furthermore, a large number of retroviruses are known that infect a wide range of eukaryotic host cells. The long terminal repeats in the retroviral DNA frequently encode functional promoters and, therefore, may be used to drive expression of the nucleic acids of the present invention.

Plasmid pRSVcat (ATCC 37152) comprises portions of a long terminal repeat of the Rous Sarcoma virus, a virus known to infect chickens and other host cells. This long terminal repeat contains a promoter which is suitable for use in the vectors of this invention. H. Gorman, et al., Proceedings of the National Academy of Sciences (USA), 79:6777 (1982). The plasmid pMSVi (NRRL B-15929) comprises the long terminal repeats of the Murine Sarcoma virus, a virus known to infect mouse and other host cells. The mouse metallothionein promoter has also been well characterized for use in eukaryotic host cells and is suitable for use in the expression of the nucleic acids of the present invention. The mouse metallothionein promoter is

present in the plasmid pdBPV-MMTneo (ATCC 37224) which can serve as the starting material of other plasmids of the present invention.

An especially preferred expression vector system employs one of a series of vectors containing the BK enhancer, an enhancer derived from the BK virus, a human papovavirus. The most preferred such vector systems are those which employ not only the BK enhancer but also the adenovirus-2-early region 1A (E1A) gene product. The E1A gene product (actually, the E1A gene produces two products, which are collectively referred to herein as "the E1A gene product") is an immediate-early gene product of adenovirus, a large DNA virus.

A most preferred expression vector employed in the present invention is the phd series of vectors which comprise a BK enhancer in tandem with the adenovirus late promoter to drive expression of useful products in eukaryotic host cells. The construction and method of using the phd plasmid, as well as related plasmids, are described in U.S. Patents 5,242,688, issued September 7, 1993, and 4,992,373, issued February 12, 1991, all of which are herein incorporated by reference. Escherichia coli K12 GM48 cells harboring the plasmid phd are available as part of the permanent stock collection of the Northern Regional Research Laboratory under accession number NRRL B-18525. The plasmid may be isolated from this culture using standard techniques.

The plasmid phd contains a unique BclI site which may be utilized for the insertion of the gene encoding the protein of interest. The skilled artisan understands that linkers or adapters may be employed in cloning the gene of interest into this BclI site. The phd series of plasmids functions most efficiently when introduced into a host cell which produces the E1A gene product, cell lines such as AV12-664, 293 cells, and others, described supra.

Transformation of the mammalian cells can be performed by any of the known processes including, but not limited to, the protoplast fusion method, the calcium phosphate co-precipitation method, electroporation and the like. See, e.g., J. Sambrook, et al., supra, at 3:16.30-3:16.66.

Other routes of production are well known to skilled artisans. In addition to the plasmid discussed above, it is well known in the art that some viruses are also appropriate vectors. For example, the adenovirus, the adeno-associated virus, the vaccinia virus, the herpes virus, the baculovirus,

and the rous sarcoma virus are useful. Such a method is described in U.S. Patent 4,775,624, herein incorporated by reference. Several alternate methods of expression are described in J. Sambrook, et al., supra, at 16.3-17.44.

In addition to prokaryotes and mammalian host cells, eukaryotic microbes such as yeast cultures may also be used. The imperfect fungus Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used eukaryotic microorganism, although a number of other strains are commonly available. For expression in Saccharomyces sp., the plasmid YRp7 (ATCC-40053), for example, is commonly used. See, e.g., L. Stinchcomb, et al., Nature (London), 282:39 (1979); J. Kingsman et al., Gene, 7:141 (1979); S. Tschemper et al., Gene, 10:157 (1980). This plasmid already contains the trp gene which provides a selectable marker for a mutant strain of yeast lacking the ability to grow in tryptophan.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [found on plasmid pAP12BD (ATCC 53231) and described in U.S. Patent No. 4,935,350, issued June 19, 1990, herein incorporated by reference] or other glycolytic enzymes such as enolase [found on plasmid pAC1 (ATCC 39532)], glyceraldehyde-3-phosphate dehydrogenase [derived from plasmid pHcGAPC1 (ATCC 57090, 57091)], hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase, as well as the alcohol dehydrogenase and pyruvate decarboxylase genes of Zymomonas mobilis (United States Patent No. 5,000,000 issued March 19, 1991, herein incorporated by reference).

Other yeast promoters, which are inducible promoters, having the additional advantage of their transcription being controllable by varying growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein [contained on plasmid vector pCL28XhoLHBPV (ATCC 39475) and described in United States Patent No. 4,840,896, herein incorporated by reference], glyceraldehyde 3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose [e.g. GAL1 found on plasmid pRY121 (ATCC 37658)] utilization. Suitable vectors and promoters for use in yeast expression are further described in R.

Hitzeman et al., European Patent Publication No. 73,657A. Yeast enhancers such as the UAS Gal from Saccharomyces cerevisiae (found in conjuction with the CYC1 promoter on plasmid YEpsec--hI1beta ATCC 67024), also are advantageously used with yeast promoters.

Practitioners of this invention realize that, in addition to the above-mentioned expression systems, the cloned cDNA may also be employed in the production of transgenic animals in which a test mammal, usually a mouse, in which expression or overexpression of the proteins of the present invention can be assessed. The nucleic acids of the present invention may also be employed in the construction of "knockout" animals in which the expression of the native cognate of the gene is suppressed.

Skilled artisans also recognize that some alterations of SEQ ID NO:2 will fail to change the function of the amino acid compound. For instance, some hydrophobic amino acids may be exchanged for other hydrophobic amino acids. Those altered amino acid compounds which confer substantially the same function in substantially the same manner as the exemplified amino acid compound are also encompassed within the present invention. Typical such conservative substitutions attempt to preserve the: (a) secondary or tertiary structure of the polypeptide backbone; (b) the charge or hydrophobicity of the residue; or (c) the bulk of the side chain. Some examples of such conservative substitutions of amino acids, resulting in the production of proteins which are functional equivalents of the protein of SEQ ID NO:2 are shown in Table II, infra.

Table II

Original Residue	Exemplary Substitutions
Ala	Ser, Gly
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro, Ala
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Mel	Leu, Ile
Phe	Met, Leu, Gyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

These substitutions may be introduced into the protein in a variety of ways, such as during the chemical synthesis or by chemical modification of an amino acid side chain after the protein has been prepared.

Alterations of the protein having a sequence which corresponds to the sequence of SEQ ID NO:2 may also be induced by alterations of the nucleic acid compounds which encodes these proteins. These mutations of the nucleic acid compound may be generated by either random mutagenesis techniques, such as those techniques employing chemical mutagens, or by site-specific mutagenesis employing oligonucleotides. Those nucleic acid compounds which confer substantially the same function in substantially the same manner as the exemplified nucleic acid compounds are also encompassed within the present invention.

Other embodiments of the present invention are nucleic acid compounds which comprise isolated nucleic acid sequences which encode SEQ ID NO:2. As skilled artisans will recognize, the amino acid compounds of the invention can be encoded by a multitude of different nucleic acid sequences because most of the amino acids are encoded by more than one nucleic acid triplet due to the degeneracy of the amino acid code. Because these alternative nucleic acid sequences would encode the same amino acid sequences, the present invention further comprises these alternate nucleic acid sequences.

The gene encoding the rhesus monkey Y2 receptor molecule may be produced using synthetic methodology. This synthesis of nucleic acids is well known in the art. See, e.g., E.L. Brown, R. Belagaje, M.J. Ryan, and H.G. Khorana, Methods in Enzymology, 68:109-151 (1979). The DNA segments corresponding to the receptor gene are generated using conventional DNA synthesizing apparatus such as the Applied Biosystems Model 380A or 380B DNA synthesizers (commercially available from Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404) which employ phosphoramidite chemistry. In the alternative, the more traditional phosphotriester chemistry may be employed to synthesize the nucleic acids of this invention. See, e.g., M.J. Gait, ed., OLIGONUCLEOTIDE SYNTHESIS, A PRACTICAL APPROACH, (1984).

The synthetic rhesus monkey Y2 receptor gene may be designed to possess restriction endonuclease cleavage sites at either end of the transcript to facilitate isolation from and integration into expression and amplification plasmids. The choice of restriction sites are chosen so as to properly orient the coding sequence of the receptor with control sequences to achieve proper in-frame reading and expression of the Y2 receptor molecule. A variety of other such cleavage sites may be incorporated depending on the particular plasmid constructs employed and may be generated by techniques well known in the art.

In an alternative methodology, the desired DNA sequences can be generated using the polymerase chain reaction as described in U.S. Patent No. 4,889,818, which is herein incorporated by reference.

In addition to the deoxyribonucleic acid of SEQ ID NO:1, this invention also provides ribonucleic acids (RNA) which comprise the RNA sequence

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   1  UGAUUGAGGU ACAAGUUGUA GACUCUUGUG CUGGUUGCAG GCCAAGUGGA
  51  ACUGUACUGA AAAUGGGUCC AAUAGGUACA GAGGCUGAUG AGAACCAGAC
 101  AGUGGAAGAA AUGAAGGUGG AACAAUAUGG GCCACAAACC ACUCCUAGAG
 151  GUGAACUGGU CCCUGAUCCU GAGCCAGAGC UUAUAGAUAG UACCAAGCUG
 201  AUUGAGGUAC AAGUUGUCCU CAUAUUGGCC UAUUGCUCCA UCAUCUUGCU
 251  UGGGGUAAUU GGCAACUCCU UGGUGAUCCA CGUGGUGAUC AAAUUCAAGA
 301  GCAUGCGCAC AGUAACCAAC UUUUUCAUCG CCAAUCUGGC UGUGGCAGAU
 351  CUUGUGGUGA AUACUCUGUG UCUACCAUUC ACUCUUACCU ACACCUUAAU
 401  GGGGGAGUGG AAAAUGGGUC CUGUCCUGUG CCACCUGGUG CCCUAUGCAC
 451  AGGGCCUGGC AGUACAAGUA UCCACAAUCA CCUUGACAGU AAUUGCCCUG
 501  GACCGGCACA GGUGCAUCGU CUACCACCUG GAGAGCAAGA UCUCCAAGCG
 551  UAUCAGCUUC CUGAUUAUUG GCUUGGCCUG GGGCAUCAGU GCCCUGCUAG
 601  CAAGUCCCCU GGCCAUCUUC CGGGAGUAUU CACUGAUUGA GAUCAUUCCG
 651  GAUUUUGAGA UUGUGGCCUG UACUGAAAAA UGGCCUGGCG AGGAAAAGAG
 701  CAUCUAUGGC ACUGUCUACA GUCUUUCUUC CUUGUUGAUC CUGUACGUUU
 751  UGCCUCUGGG CAUAAUAUCA UUUUCCUACA CUCGCAUUUG GAGUAAAUUG
 801  AAGAGCCAUG UCAGUCCUGG AGCUGCAAAU GACCACUACC AUCAGCGAAG
 851  GCAAAAAACC ACCAAAAUGC UGGUGUGCGU GGUGGUGGUG UUUGCGGUCA
 901  GCUGGCUGCC UCUCCAUGCC UUCCAGCUUG CCGUUGACAU UGACAGCCAU
 951  GUCCUGGACC UGAAGGAGUA CAAACUCAUC UUCACAGUGU UCCACAUCAU
1001  CGCCAUGUGC UCCACUUUUG CCAAUCCCCU UCUCUAUGGC UGGAUGAACA
1051  GCAACUAUAG AAAGGCUUUC CUCUCUGCCU UCCGCUGUGA GCAGCGGUUG
1101  GAUGCCAUUC ACUCUGAGGU GUCCGUGACA UUCAAGGCUA AAAAGAACCU
1151  GGAGGUCAGA AAAAAUAGUG GCCCCAAUGA CUCUUUCACA GAAGCUACCA
1201  AUGUCUAAGG AAGCUAGGGU GUGAAAAUGU AUGAAUGAAU UCUGACCAGA
1251  GCUAUAAAUC UGGUUGAUGG CGGCUCACAA GUGAUAAUUG AUUUCCCAUU
1301  UUAAGGAAGA AGAGGAUCUA AAUGGAAGCA UCUGCUGUUU AGUUCCUGGA
1351  AAACUGGCUG GGAAGAGCCU GUGUGAAAAU ACUUGAAUUC AAAGAUAAGG
1401  CAGCAAAAUG GUUUACUUAA CAGUUGGUAG GGUAGUAGGU UGAAUUAGGA
1451  GUAAAAGCAG AGAGAGGUAC UUUUGACUAU UUUCCUGGAG UGAAGUAAAC
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1501 UUGAACAAGG AAUUGGUAUU AUCAGCAUUG CAAAGAGACG GUGGGUAAAU

1551 AAGUUGAUUU UCAGAUUUCA UUAGGACCUG GAUUGGGGAG CUGUGUAGUU

1601 CACGGUUCCC UGCUUGGCUG AUGAAAACGU CGCUGAACAA AAAUUUCUCC

1651 AGGGAGCCAC AGGCUCUCCU UCAUCACGUU UUGAUUUUUU UUGUUAAUUC

1701 UCUAGACAAA AUCCAUCAAG GAAUGCUGCA GGAAAAGAUU GCCAGCUAUA

1751 UGAAUGGCUU CAAGGAACUA AACUGAAACU UGCUAUAUAA UUAAUAUUUU

1801 GGCAGACGAU AGGGGAACUC CUCAACACUC AGUGAGCCAA UUGUUCUUAA

1851 AACCGGUUGC ACAUUUGGUG AAAGUUUCUU CAACUCUGAA UCAAAAGCUG

1901 AAAUUCUCAG AAUUGCAGGA AAUGCAAACC AUCAUUUAAU UUGUAAUUUC

1951 AAGUUACAUC UGCUUUAUGG AGAUAUUUAG AUAACAAGCA UACAACUUGA

2001 UAGUUUUAUU GUUAUACCUU UUUGAACAUG UAUGAUUUAU GUUAUUAUUC

2051 CUAUUGGAGC UAAGUUUGUC UACACUAAAA UUUAAAUCAG AAUAAAGAAU

2101 AAUUUUUGUG GAAAAAAAAA AAAAAAAAAA AAAAAAAACU CGAG

hereinafter referred to as SEQ ID NO:3, or the complementary ribonucleic acid, or a fragment of either SEQ ID NO:3 or the complement thereof. The ribonucleic acids of the present invention may be prepared using the polynucleotide synthetic methods discussed supra or they may be prepared enzymatically using RNA polymerases to transcribe a DNA template. complement thereof.

The most preferred systems for preparing the ribonucleic acids of the present invention employ the RNA polymerase from the bacteriophage T7 or the bacteriophage SP6. Both of these RNA polymerases are highly specific and require the insertion of bacteriophage-specific sequences at the 5' end of the message to be read. See, J. Sambrook, et al., supra, at 18.82-18.84.

This invention also provides nucleic acids, RNA or DNA, which are complementary to SEQ ID NO:1 or SEQ ID NO:3.

The present invention also provides probes and primers useful for molecular biology techniques. A compound which encodes for SEQ ID NO:1, SEQ ID NO:3 or a complementary sequence of SEQ ID NO:1 or SEQ ID NO:3, or a fragment thereof, and which is at least 18 base pairs in length, and which will selectively hybridize to rhesus monkey genomic DNA or

messenger RNA encoding a rhesus monkey neuropeptide Y receptor, is provided. Preferably, the 18 or more base pair compound is DNA.

The term "selectively hybridize" as used herein may refer to either of two situations. In the first such embodiment of this invention, the nucleic acid compounds described supra hybridize to a rhesus monkey neuropeptide Y receptor under more stringent hybridization conditions than these same nucleic acid compounds would hybridize to an analogous neuropeptide Y receptor of another species, e.g. murine or primate. In the second such embodiment of this invention, these probes hybridize to the Y2 receptor under more stringent hybridization conditions than other related compounds, including nucleic acid sequences encoding other neuropeptide Y receptors.

These probes and primers can be prepared enzymatically as described supra. In a most preferred embodiment these probes and primers are synthesized using chemical means as described supra. Probes and primers of defined structure may also be purchased commercially.

This invention also encompasses recombinant DNA cloning vectors and expression vectors comprising the nucleic acids of the present invention. Many of the vectors encompassed within this invention are described above. The preferred nucleic acid vectors are those which are DNA. The most preferred recombinant DNA vector comprises the isolated DNA sequence SEQ ID NO:1.

Any plasmid comprising the gene of the present invention is readily modified to construct expression vectors that produce Y2 receptors in a variety of organisms, including, for example, E. coli, Sf9 (as host for baculovirus), Spodoptera and Saccharomyces. The current literature contains techniques for constructing AV12 expression vectors and for transforming AV12 host cells. United States Patent No. 4,992,373, herein incorporated by reference, is one of many references describing these techniques.

One of the most widely employed techniques for altering a nucleic acid sequence is by way of oligonucleotide-directed site-specific mutagenesis. B. Comack, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, 8.01-8.5.9, (F. Ausubel, et al., eds. 1991). In this technique an oligonucleotide, whose sequence contains the mutation of interest, is synthesized as described supra. This oligonucleotide is then hybridized to a

template containing the wild-type sequence. In a most preferred embodiment of this technique, the template is a single-stranded template. Particularly preferred are plasmids which contain regions such as the f1 intergenic region. This region allows the generation of single-stranded templates when a helper phage is added to the culture harboring the "phagemid".

After the annealing of the oligonucleotide to the template, a DNA-dependent DNA polymerase is then used to synthesize the second strand from the oliognucleotide, complementary to the template DNA. The resulting product is a heteroduplex molecule containing a mismatch due to the mutation in the oligonucleotide. After DNA replication by the host cell a mixture of two types of plasmid are present, the wild-type and the newly constructed mutant. This technique permits the introduction of convenient restriction sites such that the coding sequence may be placed immediately adjacent to whichever transcriptional or translational regulatory elements are employed by the practitioner.

The construction protocols utilized for E. coli can be followed to construct analogous vectors for other organisms, merely by substituting, if necessary, the appropriate regulatory elements using techniques well known to skilled artisans.

Host cells which harbor the nucleic acids provided by the present invention are also provided. A preferred host cell is an Xenopus sp. oocyte which has been injected with RNA or DNA compounds of the present invention. Most preferred oocytes of the present invention are those which harbor a sense mRNA of the present invention. Other preferred host cells include AV12 and E. coli cells which have been transfected and/or transformed with a vector which comprises a nucleic acid of the present invention.

The present invention also provides a method for constructing a recombinant host cell capable of expressing SEQ ID NO:2, said method comprising transforming a host cell with a recombinant DNA vector that comprises an isolated DNA sequence which encodes SEQ ID NO:2. The preferred host cell is AV12. The preferred vector for expression is one which comprises SEQ ID NO:1. Another preferred host cell for this method is E. coli. An especially preferred expression vector in E. coli is one which comprises SEQ ID NO:1. Transformed host cells may be cultured under

conditions well known to skilled artisans such that SEQ ID NO:2 is expressed, thereby producing the Y2 receptor in the recombinant host cell.

The ability of neuropeptide Y, pancreatic polypeptide, and peptide YY, to bind to the Y2 receptor is essential in the development of a multitude of indications. In developing agents which act as antagonists or agonists of the Y2 receptor, it would be desirable, therefore, to determine those agents which bind the Y2 receptor. Generally, such an assay includes a method for determining whether a substance is a functional ligand of the Y2 receptor, said method comprising contacting a functional compound of the Y2 receptor with said substance, monitoring binding activity by physically detectable means, and identifying those substances which effect a chosen response. Preferably, the physically detectable means is competition with labeled neuropeptide Y (or pancreatic polypeptide, or peptide YY) or binding of ligand in an oocyte transient expression system

The instant invention provides such a screening system useful for discovering agents which compete with neuropeptide Y for binding to the Y2 receptor, said screening system comprising the steps of:

a) isolating a rhesus monkey Y2 receptor;

b) exposing said rhesus monkey Y2 receptor to a potential inhibitor or surrogate of the neuropeptide Y/Y2 receptor complex;

c) introducing neuropeptide Y (or pancreatic polypeptide or peptide YY);

d) removing non-specifically bound molecules; and

e) quantifying the concentration of bound potential inhibitor and/or neuropeptide Y (or pancreatic polypeptide or peptide YY).

This allows one to rapidly screen for inhibitors or surrogates of the formation of the neuropeptide Y/Y2 receptor complex. Utilization of the screening system described above provides a sensitive and rapid means to determine compounds which interfere with the formation of the neuropeptide Y/Y2 receptor complex. This screening system may also be adapted to automated procedures such as a PANDEX® (Baxter-Dade Diagnostics)

system allowing for efficient high-volume screening of potential therapeutic agents.

In the assay supra, as well those infra, the neuropeptide Y employed therein may be replaced with pancreatic polypeptide or peptide YY. The neuropeptide Y used as a ligand is, therefore, merely illustrative and is not to be considered limiting in any way.

In such a screening protocol a Y2 receptor is prepared as elsewhere described herein, preferably using recombinant DNA technology. A sample of a test compound is then introduced to the reaction vessel containing the Y2 receptor followed by the addition of neuropeptide Y (or pancreatic polypeptide or peptide YY). In the alternative the neuropeptide Y (or pancreatic polypeptide or peptide YY) may be added simultaneously with the test compound. Unbound molecules are washed free and the eluent inspected for the presence of neuropeptide Y (or pancreatic polypeptide or peptide YY) or the test compound.

For example, in a preferred method of the invention, radioactively or chemically labeled neuropeptide Y (or pancreatic polypeptide or peptide YY) may be used. The eluent is then scored for the chemical label or radioactivity. The absence or diminution of the chemical label or radioactivity indicates the formation of the neuropeptide Y/Y2 receptor complex. This indicates that the test compound has not effectively competed with neuropeptide Y in the formation of the neuropeptide Y/Y2 receptor complex. The presence of the chemical label or radioactivity indicates that the test compound has competed with neuropeptide Y in the formation of the neuropeptide Y/Y2 receptor complex. Similarly, a radioactively or chemically labeled test compound may be used in which case the same steps as outlined above would be used except that the interpretation of results would be the converse of using radioactively or chemically labelled neuropeptide Y.

As would be understood by the skilled artisan these assays may also be performed such that the practitioner measures the radioactivity or fluorescence remaining with the protein, not in the eluent. A preferred such assay employs radiolabeled neuropeptide Y (or pancreatic polypeptide or peptide YY). After the competition reaction has been performed the reaction mixture is then passed through a filter, the filter retaining the receptor and whatever is complexed with the receptor. The radioactivity on each filter is then measured in a scintillation counter. In such an assay higher amounts of

radiolabel present indicate lower affinity for the receptor by the test compound.

The Y2 receptor may be free in solution or bound to a solid support. Whether the Y2 receptor is bound to a support or is free in solution, it is generally important that the conformation of the protein be conserved. In a preferred practice of the invention, therefore, the Y2 receptor is suspended in a hydrophobic environment employing natural or synthetic detergents, membrane suspensions, and the like. Preferred detergent complexes include the zwitterionic detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate ("CHAPS") as well as sodium deoxycholate.

Skilled artisans will recognize that desirable dissociation constant (K_i) values are dependent on the selectivity of the compound tested. For example, a compound with a K_i which is less than 10 nM is generally considered an excellent candidate for drug therapy. However, a compound which has a lower affinity, but is selective for the particular receptor, may be an even better candidate. The present invention, however, provides radiolabeled competition assays, whether results therefrom indicate high affinity or low affinity to Y2 receptor, because skilled artisans will recognize that any information regarding binding or selectivity of a particular compound is beneficial in the pharmaceutical development of drugs.

Assays useful for evaluating neuropeptide Y receptor antagonists are well known in the art. See, e.g., United States Patents 5,284,839, issued February 8, 1994, which is herein incorporated by reference. See also, M.W. Walker, et al., Journal of Neurosciences, 8:2438-2446 (1988).

Transient transfection protocol

Cos-1 cells are seeded at a density of 10^5 cells/150 mm dish on day one. On day three the cells are transfected (using commercially available kits) with either 25 μg rhesus monkey Y2 receptor, 50 μg rhesus monkey Y2 receptor, or 25 μg pSVLuc (SV40 Luciferase-control). Briefly, 4 ml media containing supercoiled DNA are combined with 4 ml media containing 0.6 ml of the commercial tranfection enhancing agent while mixing. This mixture is

incubated for 15 minutes at room temperature, then 16 ml of media is added to the tube and gently mixed. The cells are washed with PBS and 10 ml is added per dish. The cells are incubated at 37°C for 6 hours and 10 ml of media containing 20% fetal bovine serum is added. On day 5 (48 hrs post-transfection) the cells are scraped into phosphate buffered saline, pelleted, and kept on ice until binding assays are performed.

Stable transfection of CHO cells

A vector containing the Y2 receptor insert, is linearized using and transfected into Chinese hamster ovary (CHO) cells using commercially available reagents. The cells are maintained under 5% carbon dioxide in Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F-12 Medium (3:1) containing 10% fetal bovine serum, 2 mM glutamine, 100 international units of penicillin, and 100 μg/ml streptomycin. Stably transfected cells are selected with 500 μg/ml G418 and tested for their ability to bind [^{125}I]-PYY, infra.

[^{125}I]-PYY binding protocol

The homogenate binding studies are conducted using known methods. See, e.g., D.R. Gehlert, et al., Neurochemistry International, 21: 45-67 (1992). The cell pellets are resuspended using a glass homogenizer in 25 mM HEPES (pH 7.4) buffer containing 2.5 mM calcium chloride, 1 mM magnesium chloride and 2 g/l Bacitracin. Incubations are performed in a final volume of 200 μl containing various concentrations [^{125}I]-PYY (SA 2200 Ci/mmol) or [^{125}I]-bPP (SA 2000 Ci/mmol) and 0.2-0.4 mg protein for 2 hours at room temperature. Nonspecific binding is defined as the amount of radioactivity remaining bound to the tissue after incubating in the presence of 1 μM hPP.

In pharmacological studies, various concentrations of peptides are included in the incubation mixture. Saturation experiments are performed with each radioligand by incubating in various concentrations of the radioligand in the assay. Incubations are terminated by rapid filtration through glass fiber filters, which had been presoaked in 0.3% polyethyleneimine, using a cell harvester. The filters are washed with 5 ml

of 50 mM Tris (pH 7.4) at 4°C and rapidly dried at 60°C. The dried filters are treated with melt-on scintillator sheets, and the radioactivity retained on the filters are counted. The results are analyzed using the Lundon-1 software package (Lundon Inc., Chagrin Falls, Ohio) running on a VAX computer or the Cheng-Prushoff equation. Protein concentrations are measured using standard staining techniques, using bovine serum albumin for standards.

In one such competition assay, a battery of known neuropeptide Y receptor antagonists, agonists, and partial agonists are evaluated for their relative abilities to inhibit the binding of [^{125}I]peptide YY to the rhesus monkey Y2 receptor of the present invention.

The previously described screening systems identify compounds which competitively bind to the Y2 receptor. Determination of the ability of such compounds to stimulate or inhibit the action of the Y2 receptor is essential to further development of such compounds for therapeutic applications. The need for a bioactivity assay system which determines the response of the Y2 receptor to a compound is clear. The instant invention provides such a bioactivity assay, said assay comprising the steps of:

a) transfecting a mammalian host cell with an expression vector comprising DNA encoding a Y2 receptor;

b) culturing said host cell under conditions such that the DNA encoding the Y2 receptor is expressed,

c) exposing said host cell so transfected to a test compound, and

d) measuring the change in a physiological condition known to be influenced by the binding of neuropeptide Y to the Y2 receptor relative to a control in which the transfected host cell is exposed to neuropeptide Y.

An oocyte transient expression system can be constructed according to the procedure described in S. Lübbert, et al., Proceedings of the National Academy of Sciences (USA), 84:4332 (1987).

In an especially preferred embodiment of this invention an assay measuring the inhibition of forskolin-stimulated cAMP synthesis is performed. The inhibition of cAMP synthesis is known to positively correlated with the addition of neuropeptide Y to cells containing certain types of neuropeptide Y receptors.

Adenylate Cyclase Activity.

Adenylate cyclase activity is determined in initial experiments in transfected mammalian cells, using standard techniques. See, e.g., N. Adham, et al., supra,; R.L. Weinshank, et al., Proceedings of the National Academy of Sciences (USA), 89:3630-3634 (1992), and the references cited therein.

Adenylate cyclase activity is measured using CHO cells stably transfected with the Y2 receptor and prelabelled with 2μCi/ml of [^{3}H]-adenine for three hours. The media is removed and replaced with incubation media containing a Tyrode-HEPES buffer, 100 μM Rolipram, 10 μM indolidan, 10 μM phosphoramidon and 12.6 mg bacitracin/100 ml. Adenylate cyclase activity is increased by the addition of 15 μM forskolin and the ability of the peptide to inhibit this activation is measured using various concentrations. Following the incubation, the [^{3}H]-cAMP is separated by chromatography on alumina columns with [^{32}P]-cAMP as an internal standard. The results are quantitated by dual channel scintillation counting.

In another embodiment of this invention an assay which correlates neuropeptide Y activity with the hydrolysis of phosphatidylinositol is performed. The hydrolysis of phosphatidylinositol is known to positively correlate with addition of neuropeptide Y. This biochemical assay is performed essentially as described by M. Berridge, Biochemistry Journal, 212:849 (1983).

Phosphatidylinositol Assay

Twenty-four-well tissue-culture vessels are seeded with approximately 250,000 cells per well in Dulbecco's Minimal Essential Media (D-MEM) (in the absence of glutamic acid) which contained 2 mM glutamine

and 10% dialyzed fetal calf serum. After 24 hours growth at 37°C the media was removed and replaced with fresh media containing four microcuries of [^{3}H]myoinositol per well and the cultures are incubated a further 16 to 20 hours. The media was then removed and the cells in each well are washed with serum free medium containing 10 mM lithium chloride, 10 mM myoinositol, and 10 mM HEPES (2 x 1 ml washes). After the final wash, 0.5 ml of washing solution was added containing the appropriate concentrations fo drugs and vehichles.

If the particular assay was testing antagonists, a ten minute incubation was performed prior to agonist induction. Cells are incubated for about one hour at 37°C in 95%/5% O_2/CO_2 or as appropriate for time course. The reactions are terminated by removing media and adding 1 ml of coled 1:1 acetone:methanol followed by induction on ice for a minimum of twenty minutes.

These extracts are then removed and placed in 1.5 ml centrifuge tubes. Each well was washed with 0.5 ml water and this wash was added to the appropriate extract. After mixing and centrifugation, each aqueous supernatant was processed by chromatography on a QMA SEP-PAK® column, which had previously been wetted and equilibrated by passing 10 ml of water, followed by 8 ml of 1 M triethylammonium hydrogen carbonate (TEAB), followed by 10 ml of water through the column.

The assay supernatants containing the water soluble [^{3}H]inositol phosphate are passed over the columns. This was followed by a 10 ml water wash and a 4 ml wash with 0.02 MTEAB to remove [^{3}H]inositol precursors. [^{3}H]Inositol phosphate was eluted with 4 ml of 0.1 M TEAB into scintillation vials and counted in the presence of scintillation cocktail. Total protein in each sample was measured using standard techniques. Assays are measured as the amount of [^{3}H]inositol phosphate release per milligram of protein.

In another embodiment this invention provides a method for identifying, in a test sample, DNA homologous to a probe of the present invention, wherein the test nucleic acid is contacted with the probe under hybridizing conditions and identified as being homologous to the probe. Hybridization techniques are well known in the art. See, e.g., J. Sambrook, et al., supra, at Chapter 11.

The nucleic acid compounds of the present invention may also be used to hybridize to genomic DNA which has been digested with one or more restriction enzymes and run on an electrophoretic gel. The hybridization of radiolabeled probes onto such restricted DNA, usually fixed to a membrane after electrophoresis, is well known in the art. See, e.g., J. Sambrook, supra. Such procedures may be employed in searching for persons with mutations in these receptors by the well-known techniques of restriction fragment length polymorphisms (RFLP), the procedures of which are described in U.S. Patent 4,666,828, issued May 19, 1987, the entire contents of which is herein incorporated by reference.

The proteins of this invention as well as fragments of these proteins may be used as antigens for the synthesis of antibodies. The term "antibody" as used herein describes antibodies, fragments of antibodies (such as, but not limited, to Fab, Fab', Fab_2', and Fv fragments), and chimeric, humanized, veneered, resurfaced, or CDR-grafted antibodies capable of binding antigens of a similar nature as the parent antibody molecule from which they are derived. The instant invention also encompasses single chain polypeptide binding molecules.

The term "antibody" as used herein is not limited by the manner in which the antibodies are produced, whether such production is in situ or not. The term "antibody" as used in this specification encompasses those antibodies produced by recombinant DNA technology means including, but not limited, to expression in bacteria, yeast, insect cell lines, or mammalian cell lines.

The production of antibodies, both monoclonal and polyclonal, in animals, especially mice, is well known in the art. See, e.g., C. Milstein, HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, (Blackwell Scientific Pub., 1986); J. Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE, (Academic Press, 1983). For the production of monoclonal antibodies the basic process begins with injecting a mouse, or other suitable animal, with an immunogen. The mouse is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells, resulting in a hybridoma that reproduces in vitro. The population of hybridomas is screened to isolate individual clones, each of which secretes a single antibody species, specific for the immunogen. The individual antibody species obtained in this way is each the product of a single B cell from the immune animal generated in response

to a specific antigenic site, or epitope, recognized on the immunogenic substance.

Chimeric antibodies are described in U.S. Patent No. 4,816,567, which issued March 28, 1989 to S. Cabilly, et al. This reference discloses methods and vectors for the preparation of chimeric antibodies. The entire contents of U.S. Patent No. 4,816,567 are herein incorporated by reference. An alternative approach to production of genetically engineered antibodies is provided in U.S. Patent No. 4,816,397, which also issued March 28, 1989 to M. Boss, et al., the entire contents of which are herein incorporated by reference. The Boss patent teaches the simultaneous co-expression of the heavy and light chains of the antibody in the same host cell.

The approach of U.S. Patent 4,816,397 has been further refined as taught in European Patent Publication No. 0 239 400, which published September 30, 1987. The teachings of this European patent publication (Winter) are a preferred format for the genetic engineering of the reactive monoclonal antibodies of this invention. The Winter technology involves the replacement of complementarity determining regions (CDRs) of a human antibody with the CDRs of a murine monoclonal antibody thereby converting the specificity of the human antibody to the specificity of the murine antibody which was the source of the CDR regions. This "CDR grafting" technology affords a molecule containing minimal murine sequence and thus is less immunogenic.

Single chain antibody technology is yet another variety of genetically engineered antibody which is now well known in the art. See, e.g. R.E. Bird, et al., Science 242:423-426 (1988); PCT Publication No. WO 88/01649, which was published 10 March 1988; United States Patent 5,260,203, issued November 9, 1993, the entire contents of which are herein incorporated by reference. The single chain antibody technology involves joining the binding regions of heavy and light chains with a polypeptide sequence to generate a single polypeptide having the binding specificity of the antibody from which it was derived.

The aforementioned genetic engineering approaches provide the skilled artisan with numerous means to generate molecules which retain the binding characteristics of the parental antibody while affording a less immunogenic format.

These antibodies are used in diagnostics, therapeutics or in diagnostic/therapeutic combinations. By "diagnostics" as used herein is meant testing that is related to either the in vitro or in vivo diagnosis of disease states or biological status in mammals, preferably in humans. By "therapeutics" and "therapeutic/diagnostic combinations" as used herein is respectively meant the treatment or the diagnosis and treatment of disease states or biological status by the in vivo administration to mammals, preferably humans, of the antibodies of the present invention. The antibodies of the present invention are especially preferred in the diagnosis and/or treatment of conditions associated with an excess or deficiency of Y2 receptors.

In addition to being functional as direct therapeutic and diagnostic aids, the availability of a family of antibodies which are specific for the Y2 receptor enables the development of numerous assay systems for detecting agents which bind to this receptor. One such assay system comprises radiolabeling Y2 receptor-specific antibodies with a radionuclide such as ^{125}I and measuring displacement of the radiolabeled Y2 receptor-specific antibody from solid phase Y2 receptor in the presence of a potential antagonist.

Numerous other assay systems are also readily adaptable to detect agents which bind Y2 receptor. Examples of these aforementioned assay systems are discussed in Methods in Enzymology, (J. Langone. and H. Vunakis, eds. 1981), Vol. 73, Part B, the contents of which are herein incorporated by reference. Skilled artisans are directed to Section II of Methods in Enzymology, Vol. 73, Part B, supra, which discusses labeling of antibodies and antigens, and Section IV, which discusses immunoassay methods.

In addition to the aforementioned antibodies specific for the Y2 receptor, this invention also provides antibodies which are specific for the hypervariable regions of the anti-Y2 receptor antibodies. Some such anti-idiotypic antibodies would resemble the original epitope, the Y2 receptor, and, therefore, would be useful in evaluating the effectiveness of compounds which are potential antagonists, agonists, or partial agonists of the Y2 receptor. See, e.g., Cleveland, et al., Nature (London), 305:56 (1983); Wasserman, et al., Proceedings of the National Academy of Sciences (USA), 79:4810 (1982).

In another embodiment, this invention encompasses pharmaceutical formulations for parenteral administration which contain, as the active ingredient, the anti-Y2 receptor antibodies described, supra. Such formulations are prepared by methods commonly used in pharmaceutical chemistry.

Products for parenteral administration are often formulated and distributed in solid, preferably freeze-dried form, for reconstitution immediately before use. Such formulations are useful compositions of the present invention. Their preparation is well understood by pharmaceutical chemists.

In general, these formulations comprise the active ingredient in combination with a mixture of inorganic salts, to confer isotonicity, as well as dispersing agents such as lactose, to allow the dried preparation to dissolve quickly upon reconstitution. Such formulations are reconstituted for use with highly purified water to a known concentration.

Alternatively, a water soluble form of the antibody can be dissolved in one of the commonly used intravenous fluids and administered by infusion. Such fluids include physiological saline, Ringer's solution or a 5% dextrose solution.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Baez, Melvyn
Yang, Peiyi

(ii) TITLE OF INVENTION: RHESUS MONKEY NEUROPEPTIDE Y RECEPTOR

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Indianapolis
(D) STATE: Indiana
(E) COUNTRY: United States of America
(F) ZIP: 46285

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: P-10901

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2144 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 63..1205

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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     1               5                  10                  15

GAA ATG AAG GTG GAA CAA TAT GGG CCA CAA ACC ACT CCT AGA GGT GAA         155
Glu Met Lys Val Glu Gln Tyr Gly Pro Gln Thr Thr Pro Arg Gly Glu
                 20                  25                  30

CTG GTC CCT GAT CCT GAG CCA GAG CTT ATA GAT AGT ACC AAG CTG ATT         203
Leu Val Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Ile
             35                  40                  45

GAG GTA CAA GTT GTC CTC ATA TTG GCC TAT TGC TCC ATC ATC TTG CTT         251
Glu Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu
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GGG GTA ATT GGC AAC TCC TTG GTG ATC CAC GTG GTG ATC AAA TTC AAG         299
Gly Val Ile Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys
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AGC ATG CGC ACA GTA ACC AAC TTT TTC ATC GCC AAT CTG GCT GTG GCA         347
Ser Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala
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GAT CTT GTG GTG AAT ACT CTG TGT CTA CCA TTC ACT CTT ACC TAC ACC         395
Asp Leu Val Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr
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Leu Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro
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TAT GCA CAG GGC CTG GCA GTA CAA GTA TCC ACA ATC ACC TTG ACA GTA         491
Tyr Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val
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Leu Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr
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ACT CGC ATT TGG AGT AAA TTG AAG AGC CAT GTC AGT CCT GGA GCT GCA      827
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AAT GAC CAC TAC CAT CAG CGA AGG CAA AAA ACC ACC AAA ATG CTG GTG      875
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TGC GTG GTG GTG GTG TTT GCG GTC AGC TGG CTG CCT CTC CAT GCC TTC      923
Cys Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe
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CAG CTT GCC GTT GAC ATT GAC AGC CAT GTC CTG GAC CTG AAG GAG TAC      971
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        290                 295                 300

AAA CTC ATC TTC ACA GTG TTC CAC ATC ATC GCC ATG TGC TCC ACT TTT     1019
Lys Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe
    305                 310                 315

GCC AAT CCC CTT CTC TAT GGC TGG ATG AAC AGC AAC TAT AGA AAG GCT     1067
Ala Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala
320                 325                 330                 335

TTC CTC TCT GCC TTC CGC TGT GAG CAG CGG TTG GAT GCC ATT CAC TCT     1115
Phe Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser
                340                 345                 350

GAG GTG TCC GTG ACA TTC AAG GCT AAA AAG AAC CTG GAG GTC AGA AAA     1163
Glu Val Ser Val Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Arg Lys
            355                 360                 365

AAT AGT GGC CCC AAT GAC TCT TTC ACA GAA GCT ACC AAT GTC             1205
Asn Ser Gly Pro Asn Asp Ser Phe Thr Glu Ala Thr Asn Val
```

370 375 380

TAAGGAAGCT AGGGTGTGAA AATGTATGAA TGAATTCTGA CCAGAGCTAT AAATCTGGTT 1265

GATGGCGGCT CACAAGTGAT AATTGATTTC CCATTTTAAG GAAGAAGAGG ATCTAAATGG 1325

AAGCATCTGC TGTTTAGTTC CTGGAAAACT GGCTGGGAAG AGCCTGTGTG AAAATACTTG 1385

AATTCAAAGA TAAGGCAGCA AAATGGTTTA CTTAACAGTT GGTAGGGTAG TAGGTTGAAT 1445

TAGGAGTAAA AGCAGAGAGA GGTACTTTTG ACTATTTTCC TGGAGTGAAG TAAACTTGAA 1505

CAAGGAATTG GTATTATCAG CATTGCAAAG AGACGGTGGG TAAATAAGTT GATTTTCAGA 1565

TTTCATTAGG ACCTGGATTG GGGAGCTGTG TAGTTCACGG TTCCCTGCTT GGCTGATGAA 1625

AACGTCGCTG AACAAAAATT TCTCCAGGGA GCCACAGGCT CTCCTTCATC ACGTTTTGAT 1685

TTTTTTTGTT AATTCTCTAG ACAAAATCCA TCAAGGAATG CTGCAGGAAA AGATTGCCAG 1745

CTATATGAAT GGCTTCAAGG AACTAAACTG AAACTTGCTA TATAATTAAT ATTTTGGCAG 1805

ACGATAGGGG AACTCCTCAA CACTCAGTGA GCCAATTGTT CTTAAAACCG GTTGCACATT 1865

TGGTGAAAGT TTCTTCAACT CTGAATCAAA AGCTGAAATT CTCAGAATTG CAGGAAATGC 1925

AAACCATCAT TTAATTTGTA ATTTCAAGTT ACATCTGCTT TATGGAGATA TTTAGATAAC 1985

AAGCATACAA CTTGATAGTT TTATTGTTAT ACCTTTTTGA ACATGTATGA TTTATGTTAT 2045

TATTCCTATT GGAGCTAAGT TTGTCTACAC TAAAATTTAA ATCAGAATAA AGAATAATTT 2105

TTGTGGAAAA AAAAAAAAAA AAAAAAAAAA AAACTCGAG 2144

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 381 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gly Pro Ile Gly Thr Glu Ala Asp Glu Asn Gln Thr Val Glu Glu
1 5 10 15

Met Lys Val Glu Gln Tyr Gly Pro Gln Thr Thr Pro Arg Gly Glu Leu
20 25 30

35 40 45

Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly
50 55 60

Val Ile Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser
65 70 75 80

Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp
85 90 95

Leu Val Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu
100 105 110

Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr
115 120 125

Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile
130 135 140

Ala Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile
145 150 155 160

Ser Lys Arg Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Ile Ser
165 170 175

Ala Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile
180 185 190

Glu Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro
195 200 205

Gly Glu Glu Lys Ser Ile Tyr Gly Thr Val Tyr Ser Leu Ser Ser Leu
210 215 220

Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr
225 230 235 240

Arg Ile Trp Ser Lys Leu Lys Ser His Val Ser Pro Gly Ala Ala Asn
245 250 255

Asp His Tyr His Gln Arg Arg Gln Lys Thr Thr Lys Met Leu Val Cys
260 265 270

Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln
275 280 285

Leu Ala Val Asp Ile Asp Ser His Val Leu Asp Leu Lys Glu Tyr Lys
290 295 300

Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala
305 310 315 320

Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe
325 330 335

Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu
340 345 350

Val Ser Val Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Arg Lys Asn
355 360 365

Ser Gly Pro Asn Asp Ser Phe Thr Glu Ala Thr Asn Val
370 375 380

We Claim:

1. An isolated amino acid compound functional as a rhesus monkey receptor having affinity for neuropeptide Y, pancreatic polypeptide, peptide YY, and which comprises the amino acid sequence

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Met Gly Pro Ile Gly Thr Glu Ala Asp Glu Asn Gln Thr Val Glu Glu
  1               5                  10                  15

Met Lys Val Glu Gln Tyr Gly Pro Gln Thr Thr Pro Arg Gly Glu Leu
             20                  25                  30

Val Pro Asp Pro Glu Pro Glu Leu Ile Asp Ser Thr Lys Leu Ile Glu
         35                  40                  45

Val Gln Val Val Leu Ile Leu Ala Tyr Cys Ser Ile Ile Leu Leu Gly
     50                  55                  60
Val Ile Gly Asn Ser Leu Val Ile His Val Val Ile Lys Phe Lys Ser
 65                  70                  75                  80

Met Arg Thr Val Thr Asn Phe Phe Ile Ala Asn Leu Ala Val Ala Asp
                 85                  90                  95

Leu Val Val Asn Thr Leu Cys Leu Pro Phe Thr Leu Thr Tyr Thr Leu
            100                 105                 110

Met Gly Glu Trp Lys Met Gly Pro Val Leu Cys His Leu Val Pro Tyr
        115                 120                 125

Ala Gln Gly Leu Ala Val Gln Val Ser Thr Ile Thr Leu Thr Val Ile
    130                 135                 140

Ala Leu Asp Arg His Arg Cys Ile Val Tyr His Leu Glu Ser Lys Ile
145                 150                 155                 160

Ser Lys Arg Ile Ser Phe Leu Ile Ile Gly Leu Ala Trp Gly Ile Ser
                165                 170                 175

Ala Leu Leu Ala Ser Pro Leu Ala Ile Phe Arg Glu Tyr Ser Leu Ile
            180                 185                 190

Glu Ile Ile Pro Asp Phe Glu Ile Val Ala Cys Thr Glu Lys Trp Pro
        195                 200                 205

Gly Glu Glu Lys Ser Ile Tyr Gly Thr Val Tyr Ser Leu Ser Ser Leu
    210                 215                 220

Leu Ile Leu Tyr Val Leu Pro Leu Gly Ile Ile Ser Phe Ser Tyr Thr
225                 230                 235                 240

Arg Ile Trp Ser Lys Leu Lys Ser His Val Ser Pro Gly Ala Ala Asn
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                    245                 250                 255

Asp His Tyr His Gln Arg Arg Gln Lys Thr Thr Lys Met Leu Val Cys
            260                 265                 270

Val Val Val Val Phe Ala Val Ser Trp Leu Pro Leu His Ala Phe Gln
        275                 280                 285

Leu Ala Val Asp Ile Asp Ser His Val Leu Asp Leu Lys Glu Tyr Lys
    290                 295                 300

Leu Ile Phe Thr Val Phe His Ile Ile Ala Met Cys Ser Thr Phe Ala
305                 310                 315                 320

Asn Pro Leu Leu Tyr Gly Trp Met Asn Ser Asn Tyr Arg Lys Ala Phe
                325                 330                 335

Leu Ser Ala Phe Arg Cys Glu Gln Arg Leu Asp Ala Ile His Ser Glu
            340                 345                 350

Val Ser Val Thr Phe Lys Ala Lys Lys Asn Leu Glu Val Arg Lys Asn
        355                 360                 365

Ser Gly Pro Asn Asp Ser Phe Thr Glu Ala Thr Asn Val
    370                 375                 380
```

which is SEQ ID NO:2, or a fragment of at least 6 continuous amino acids thereof.

2. A nucleic acid compound encoding an amino acid compound of **Claim 1**.

3. A composition comprising an isolated nucleic acid compound containing a sequence encoding a rhesus monkey Y2 receptor or fragment thereof as claimed in **Claim 2**, wherein said sequence encoding a rhesus monkey neuropeptide receptor or fragment thereof is selected from the group consisting of:

(a) nucleotides 63 to 1205 of SEQ ID NO:1;

(b) nucleotides 63 to 1205 of SEQ ID NO:3;

(c) a nucleic acid compound complementary to (a) or (b); and

(d) a fragment of (a), (b), or (c) that is at least 18 bases in length and which will selectively hybridize to rhesus monkey genomic DNA encoding a rhesus monkey neuropeptide Y-like receptor.

4. A composition as claimed in **Claim 2** wherein the isolated nucleic acid compound is deoxyribonucleic acid.

5. A composition as claimed in **Claim 3** which is (a) or a sequence complementary to (a).

6. A composition as claimed in **Claim 2** wherein the isolated nucleic acid compound is ribonucleic acid.

7. A composition as claimed in **Claim 6** which is (b) or a fragment thereof.

8. An expression vector capable of producing a rhesus monkey Y2 receptor or a fragment thereof in a host cell which comprises a nucleic acid compound as claimed in **Claim 3** in combination with regulatory elements necesssary for expression of the nucleic acid compound in the host cell.

9. An expression vector as claimed in **Claim 8** for use in a host cell wherein the host cell is Escherichia coli.

10. An expression vector as claimed in **Claim 8** for use in a host cell wherein the host cell is a mammalian cell line.

11. An expression vector as claimed in **Claim 10** which further comprises the BK virus enhancer.

12. An expression vector as claimed in **Claim 11** which further comprises an adenovirus late promoter.

13. A transfected host cell harboring an expression vector as claimed in **Claim 8**.

14. A transfected host cell as claimed in **Claim 13** which is transfected Escherichia coli.

15. A transfected host cell as claimed in **Claim 13** which is a transfected mammalian cell line.

16. A method of evaluating the effectiveness of a test compound for the treatment or prevention of a condition associated with a deficiency of stimulation of a receptor of SEQ ID NO:2 which method comprises:

a) introducing into a mammalian host cell an expression vector comprising DNA encoding the receptor of SEQ ID NO:2;

b) culturing said host cell under conditions such that the receptor of SEQ ID NO:2 is expressed;

c) exposing said host cell expressing the receptor of SEQ ID NO:2 to a test compound; and

d) measuring the change in a physiological condition known to be influenced by the binding of native ligand to the receptor of SEQ ID NO:2 relative to a control in which the transfected host cell is exposed to native ligand.

17. A method of evaluating the effectiveness of a test compound for the treatment or prevention of a condition associated with an excess of stimulation of a receptor of SEQ ID NO:2, which method comprises:

a) introducing into a mammalian host cell an expression vector comprising DNA encoding the receptor of SEQ ID NO:2;

b) culturing said host cell under conditions such that the receptor of SEQ ID NO:2 is expressed;

c) exposing said host cell expressing the receptor of SEQ ID NO:2 to a test compound;

d) exposing said host cell expressing the receptor of SEQ ID NO:2 to neuropeptide Y, pancreatic polypeptide, or peptide YY, simultaneously with, or following the exposure to the test compound; and

e) measuring the change in a physiological condition known to be influenced by the binding of neuropeptide Y to the receptor of SEQ ID NO:2 relative to a control in which the transfected host cell is exposed to only the neuropeptide employed in step d).

18. A method of evaluating the effectiveness of a test compound for use in the treatment or prevention of conditions associated with an excess or deficiency of stimulation of a receptor of SEQ ID NO:2 comprising the steps of:

a) isolating the receptor of SEQ ID NO:2;

b) exposing said isolated receptor of SEQ ID NO:2 to the test compound;

c) exposing the isolated receptor of SEQ ID NO:2 to neuropeptide Y, pancreatic polypeptide, or peptide YY, simultaneously with or following the introduction of the test compound;

d) removing non-specifically bound neuropeptide or test compound;

e) quantifying the concentration of test compound or neuropeptide bound to the receptor of SEQ ID NO:2; and

f) comparing the concentration of test compound or neuropeptide bound to the receptor of SEQ ID NO:2 to a control in which no test compound were added.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10048

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 530/300, 350; 435/4, 240.2, 240.27, 320.1; 514/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 350; 435/4, 240.2, 240.27, 320.1; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG (MEDLINE, EMBASE, BIOSIS, WPI, USPATFULL). author and word. search terms: e.g. neuropeptide Y; pancreatic polypeptide; peptide yy; receptor; cDNA or clone or sequence.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BLOMQVIST et al. Cloning and sequence analysis of a neuropeptide Y/peptide YY receptor Y1 cDNA from Xenopus laevis. Biochemica et Biophysica Acta. 1995, Vol. 1261, pages 439-441, see entire document.	1-7, 9-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
29 AUGUST 1997	24 SEP 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	HEATHER BAKALYAR
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10048

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6): A61K 38/00; C07K 2/00, 4/00, 5/00, 7/00, 14/00, 16/00, 17/00; C12Q 3/00; C12N 5/00, 15/00, 15/09; A01N 37/18